

13. Organic DNA Extraction

Purpose

This procedure provides a method for isolating and purifying the DNA that is contained in blood, saliva, epithelial cells, hair, soft tissue, and bone.

Background

Biological samples contain a number of substances besides DNA. DNA molecules must be separated from other cellular material before they can be examined. Cellular proteins that package and protect DNA in the environment of the cell can inhibit the ability to amplify the DNA. Therefore, organic DNA extraction methods have been developed to separate proteins and other cellular materials from the DNA molecules. Filtration devices such as the Microcon® YM-100 can be employed to concentrate and purify the DNA and further remove impurities that can cause inhibition.

Safety

1. Appropriate safety precautions should be taken and proper personal protective equipment worn when working with hazardous or potentially hazardous materials. The MSDS sheets and the laboratory safety manual should be consulted prior to using the materials in this procedure.
2. All samples can potentially present biological hazards and should be treated as such.
3. Phenol:chloroform:isoamyl alcohol is toxic. Use only in a fume hood or chemically rated biological cabinet.
4. Following DNA/protein separation, all liquid waste containing phenol:chloroform:isoamyl alcohol solution is to be disposed of in the satellite waste receptacles located in the extraction hoods. The empty tubes can then be placed in the biohazard, also located in the extraction hoods.

Materials

Digest buffer (see section on Reagent Preparation)

Proteinase K (see section on Reagent Preparation)

DTT (see section on Reagent Preparation)

Teknova TE Buffer

Phenol:chloroform:isoamyl alcohol

Microcon® YM-100 filtration devices

Microcentrifuge tubes

UV crosslinker
Pipettes
Heat block

Quality Control/Calibration

1. A reagent blank control will be included in each extraction set to test for contamination.
2. Two extraction controls should be implemented in any situation where multiple amplifications of a sample may be necessary. The second control will ensure that enough volume is available to run concurrently with the evidence samples. Both controls must be quantitated, but only one needs to be amplified at a given time. Once the first reagent blank is consumed, testing can resume with the second reagent blank. Once both reagent blanks are consumed, no further testing can be performed on the samples in that set. An exception to this rule would be for a sample that needs to be reamplified with a previously used kit and the corresponding extract control has already mirrored the sample, in terms of injection conditions, instrument model used, volume/concentration conditions, and any other material modifications.
3. The recovered volume of reagent blank control should be equal to, or less than, the recovered volume of the sample extracts. If it is not, the control can be further concentrated, or the sample volumes can be “brought up” to the volume of the control, assuming the samples have sufficient amounts of DNA. Alternatively, excess volume in the control can be corrected for during amplification by adding the control in a total recovered volume:amplification volume ratio that equals that of the sample. For example, if 15 μ L of a sample is recovered and 30 μ L of control is recovered, the volume of control added to the amplification reaction would have to be double that of the corresponding sample.
4. If a sample requires further purification at a later time, another extraction control should be initiated and carried through the process to control for the aliquot of TE buffer used.
5. Other than phenol:chloroform:isoamyl alcohol, all extraction reagents are considered critical, and are subjected to quality control testing prior to use. Refer to the DNA QA/QC Manual for procedures and acceptance criteria.
6. Microcon® YM-100 filtration devices, all microcentrifuge tubes (0.5mL, 1.5mL, 2mL), and piggyback filters must be UV cross-linked at 250,000 μ J for 12 minutes prior to use.

Procedure

Sample preparation/Organic extraction

1. The following sample types will be prepared for the organic extraction method as follows:
 - a. Liquid blood: whole liquid blood can be aliquoted into a microcentrifuge tube in volumes ranging from 5-100 μ L, depending on the age and condition of the blood.
 - b. Biological stains/tissue: cuttings measuring approximately 0.5cm x 0.5cm to 1.5cm x 1.5cm, or, ½ swab to one whole swab, should be collected from stains containing biological material (blood, saliva, epithelial cells, tissue). The amount of sample collected will be dictated by the overall size of the stain, results of presumptive and confirmatory tests, etc.
 - c. Hair: surface contaminants must be removed prior to sampling for DNA testing by submerging the hair root in ethanol or sterile water. The hair should be air-dried or dried gently with a Kim-Wipe. Care should be taken not to destroy or lose the root. Approximately 1cm of the root end should be collected for DNA testing, and if needed, a portion of the hair shaft adjacent to the hair root may be sampled as a control.
 - d. Teeth: surface contaminants must be removed prior to sampling for DNA testing by sonicating the tooth in sterile water. While sonicating, the sterile water should be replaced periodically until the water is clean. The tooth must be pulverized prior to sampling, and this may require the use of a coffee grinder. Coffee grinders are to be sterilized prior to use and are considered single-use items which should be disposed of following sample collection.. The size and condition of the tooth will dictate the amount of sample that is needed for DNA testing.
 - e. Bone: surface contamination must be removed prior to sampling for DNA testing by cleansing the bone with sterile water and/or ethanol. It may be necessary to sand the bone surface if cleansing with water or ethanol is insufficient in removing surface contamination. Next, the bone must be pulverized prior to sampling. This may involve the use of a sterile coffee grinder or Dremel tool. These implements are single-use and should be disposed of following sample collection.
2. To each tube containing a sample, pipette 400 μ L digest buffer and 12 μ L of 10 mg/mL Proteinase K solution using a fresh, sterile barrier pipette tip (alternatively, a master mix of digest buffer and proteinase K can be prepared and then aliquoted concurrently into the sample tubes). Mix gently or vortex. For reference samples, incubate at 56°C for at least 1 hour. For evidence samples, it is recommended that digestion continue for a minimum of 6 hours. Digestion may be performed overnight, but more than 24 hours is not recommended.

3. In addition to the Proteinase K solution add 16 μL 1M DTT for potential hair roots. For tissue, bone and teeth samples, additional Proteinase K (12 μL) may be added after a few hours of digest time. Depending on the condition of the bone sample, a larger amount of material may need to be sampled. In that case, increase the reagent volumes proportionally.
4. After digestion, agitate substrate (using toothpick, pipette tip, or forceps) or vortex, and then piggyback the substrate and centrifuge at 7500RPM (5500RCF) for two minutes. Alternately, the substrate may be left in the tube.
5. To the lysed and digested cells, add 400 μL buffered phenol:chloroform:isoamyl alcohol solution. Cap the tube and vortex or hand mix for 15 seconds until a milky emulsion forms.
6. Centrifuge the sample for 3 to 5 minutes at approximately 7500RPM (5500RCF) at room temperature to separate the two phases.
7. Proceed to Microcon[®] YM-100 concentration if the upper aqueous phase is clear.
8. If the aqueous phase is not clear (e.g., cloudy, dark in color or colored from dyes), then use a sterile pipette tip to transfer the upper aqueous phase to a fresh, sterile microcentrifuge tube. Repeat Steps 5 and 6 an additional 1 to 3 times, until the interface is clean and the aqueous phase is clear. The reagent blank control must also be subjected to the additional extraction. While performing the additional extractions, the lower layer may be removed and discarded, thus eliminating the need for a fresh microcentrifuge tube after the first extraction.

Microcon[®] YM-100 concentration

1. UV cross-link the Microcon[®] YM-100 components (filter and collection tubes) at 250,000 μJ for 12 minutes.
2. Assemble and appropriately label the Microcon[®] YM-100 unit.
3. Add 50 μL of TE buffer to activate the membrane of the Microcon[®] YM-100 located in the sample reservoir.
4. Add the entire (approximately 400 μL) upper aqueous phase to the membrane containing TE buffer using a sterile pipette tip or sterile transfer pipette.
5. Centrifuge the Microcon[®] YM-100 in a fixed angle rotor at 3500RPM (1500RCF) for approximately 15 minutes. The DNA sample will remain concentrated in TE buffer on the membrane of the Microcon[®] YM-100 and

molecules with molecular weights of less than about 100,000 daltons will pass through the filter. Discard the effluent in the collection tube or transfer the sample reservoir of the Microcon® YM-100 to a new collection tube.

6. Add at least 200 µL of TE buffer to the concentrated DNA solution in the upper Microcon® YM-100 reservoir.
7. Centrifuge at a speed of 3500RPM (1500RCF) for approximately 15 minutes.
8. After each wash, determine if the collection tube needs to be replaced or emptied, such that the subsequent washes of TE buffer can pass through the membrane completely without overflowing the collection tube.
9. Repeat steps 5 through 7 as needed. For liquid blood samples and reference buccal swabs, the manufacturer's recommendation of one 200 µL wash may be used. For all evidence DNA samples, multiple washes should be performed, using, at minimum, 800 µL of TE buffer in total. Samples expected to exhibit higher levels of inhibition may require more additional washes to remove impurities.
10. Add desired elution volume (25-100 µL) of TE buffer to the Microcon® YM-100 concentrator. Reconcentration may be necessary if there is a significant disparity between the recovery volume and the elution volume.
11. Invert the concentrator into a labeled, 2.0 mL new collection tube.
12. Centrifuge samples at 5000RPM (2500RCF) for 3 minutes to collect DNA.
13. Discard the concentrator. Estimate the volume of the eluate using a pipette and transfer sample to a sterilized microcentrifuge tube. Do not use the Microcon® YM-100 collection tube for long-term DNA storage.
14. Store the sample at 2 to 8°C or freeze until quantitation and/or amplification.

Limitations/Notes

1. When placing tubes in the heat block for extraction, be sure the lids are secure and completely closed. Autoclaved plastics have been known to change shape slightly when exposed to heat and the lids can unexpectedly open.
2. DNA extracted separately from the same stain or from items having the same origin (e.g., two sets of vaginal swabs from the same victim) may be combined at any point during or after the extraction is complete.

3. Centrifugation at speeds higher than listed may disrupt the membrane and allow passage of the DNA from the upper reservoir to the lower one. If this occurs, the eluant from both upper and lower reservoirs should be combined and the procedure repeated with a new device.
4. Care should be taken not to transfer the phenol:chloroform:isoamyl alcohol reagent to the Microcon® YM-100 concentrator, as it can harm the membrane and allow DNA to pass through. Residual phenol:chloroform:isoamyl alcohol can also inhibit the DNA that does remain on the membrane.

Documentation

Organic extraction worksheet

Revision history:

Revision No.	Date	Approved By	Revisions
0	11/16/2010	L Burley	Original
1	3/15/2011	L Burley	Specified that all microcentrifuge tubes and piggyback filters must be UV crosslinked prior to use.